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WATER CONTENT OF STRATUM CORNEUM 'IN VIVO'. (U)

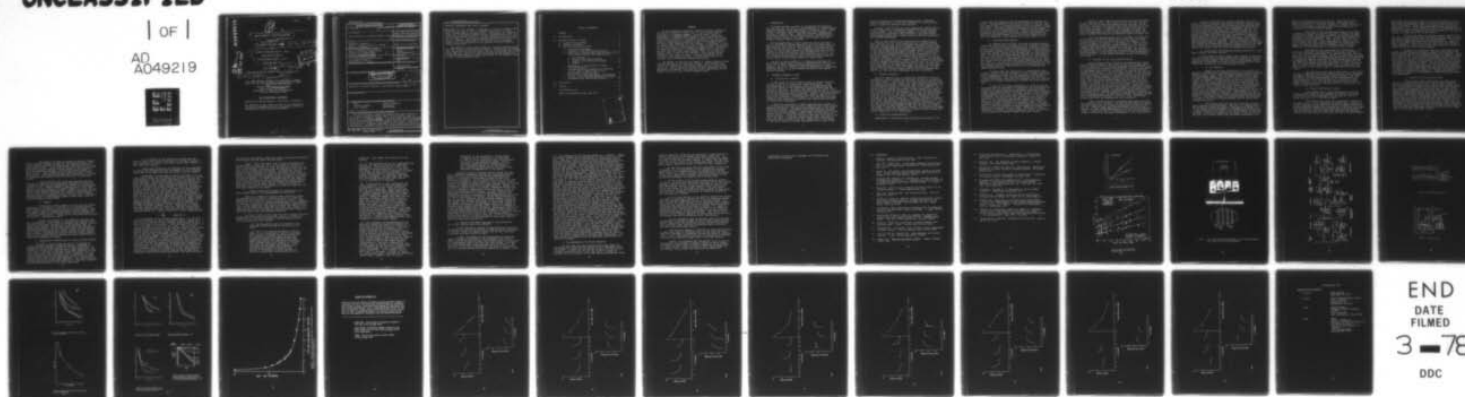
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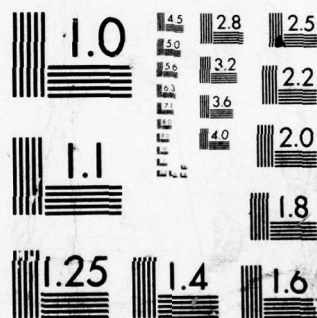
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Abstract (continued from front of page)

developed for this purpose. Calibration *in vitro* on samples taken from the same site is made by comparing resistivity vs. total water content measure gravimetrically. Differences in conduction properties of different samples are not now predictable, and can cause large estimate errors if a single calibration curve is used for all subjects. The transfer method eliminates this source of errors. Present limitations on the accuracy of the transfer method arise from uncertainties in regional water distribution during calibrations. *In theory, the*

The method is restricted ~~in theory~~ to stratum corneum thicker than the spacing of the electrode fingers. With present electrodes this theoretical restriction limits the use to the palms and soles. Future empirical testing of these electrodes and the manufacture of smaller electrodes may permit application of the method to areas of thin stratum corneum as well.

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## TABLE OF CONTENTS

Summary . . . . .	2
I. Introduction . . . . .	3
II. Research Progress to Date . . . . .	3
A. Criteria for a Method . . . . .	3
B. Thermal Constants . . . . .	4
C. Electrical Characteristics . . . . .	4
1. Instrument Development . . . . .	5
2. Accuracy of the Probe and Electronics . . . . .	6
3. Quantitative Relationships Between Resistivity, Total Water Content, and Other Factors . . . . .	7
a. Test Methods . . . . .	7
b. Results with Normal Corneum . . . . .	8
c. Results with Pretreated Corneum . . . . .	9
d. Summary . . . . .	10
4. Measurement of Resistivity of Intact Stratum Corneum . . . . .	10
5. Proposed Application Method for Inferring <i>In Vivo</i> Water Content from <i>In Vivo</i> Measurements of Resistivity . . . . .	12
6. Results From Human Experiments Illustrating the Use of the Proposed Application Methods . . . . .	14
7. Recommendations for Future Research . . . . .	15
III. References . . . . .	18
IV. Figures . . . . .	20
Distribution List . . . . .	36
Report Documentation Page (DD 1473) . . . . .	1

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## SUMMARY

We are developing a method to measure the water content of the stratum corneum of intact skin. In its present form the technique is a transfer method. Like many other transfer measurements in medicine the technique is most accurate when individually calibrated with samples of the subjects own tissue. Electrical resistivity is accurately measured *in vivo* during the course of experimental procedures using a four-fingered micro-electrode probe developed for this purpose. Calibration *in vitro* on samples taken from the same site is made by comparing resistivity vs. total water content measure gravimetrically. Differences in conduction properties of different samples are not now predictable, and can cause large estimate errors if a single calibration curve is used for all subjects. The transfer method eliminates this source of error. Present limitations on the accuracy of the transfer method arise from uncertainties in regional water distribution during calibrations.

The method is restricted in theory to stratum corneum thicker than the spacing of the electrode fingers. With present electrodes this theoretical restriction limits the use to the palms and soles. Future emperical testing of these electrodes and the manufacture of smaller electrodes may permit application of the method to areas of thin stratum corneum as well.

## I. INTRODUCTION

Prolonged exposure to water or the wearing of waterproof foot and leg wear can result in the production of incapacitating immersion disease, often complicated by infection (1-3). Under suitable conditions immersion disease can reach epidemic proportions in combat troupes. Immersion diseases, dermatophyte infections, miliaria, and friction blisters, represent occupational hazards unique to the combat foot soldier, and thus uniquely military medical problems.

The pathophysiology of water immersion injury and other water or occlusion related diseases is not well understood, but one important factor may be the breakdown of the skin barrier layer, the *stratum corneum*. The integrity and mechanical properties of the stratum corneum are dependent upon many factors, a major one being the correct level of hydration (4,5,6). At this time, we do not know what this hydration level is *in vivo*, nor has there been devised a method for accurately measuring water content in the stratum corneum of intact skin.

*The goal of these studies is to develop methodology for this purpose which could be used by researchers at LAIR or elsewhere (1) to better understand the pathophysiology of these diseases, and (2) to develop protective measures for maintaining hydration levels of stratum corneum consistent with optimal barrier function.*

## II. RESEARCH PROGRESS TO DATE

### A. Criteria for a Method

At the outset of these studies we set up certain criteria for the selection of a potential measurement method. First, the method had to be noninvasive, that is somehow measuring the water content of the underlying stratum corneum from the surface of the skin. Secondly, the method had to clearly "see" the water content of the surface keratin layer and not be influenced by the well-hydrated viable epidermis. Thirdly, the method would have to be relatively inexpensive and simple to operate, or at least have a potential for becoming so, in order that such measurements could eventually be made by less experienced personnel or outside of a laboratory.

It was first necessary to choose some physical property of water which was well-related to the amount of water, and could be measured externally. The problem was complicated by the three practical criteria listed above, and by two additional factors. First, the stratum corneum is complicated and chemically a poorly defined matrix of insoluble protein, lipid, and low molecular weight solubles. Water does not enter and leave this matrix simply; some water is trapped or bound, while other molecules of water are freer to move in and out. Differences in the makeup of the matrix material between individuals could result in dif-

ferent distributions of bound and unbound water. Measured physical properties of water would probably be affected differently by bound and unbound water fractions.

Secondly, there existed no other quantitative method which could be applied as a comparison standard for measuring water in intact skin. Even for removed samples of stratum corneum which could be processed in the laboratory, the quantitative relationship between water content and most physical properties has not been determined.

Thus, at the outset it was clear that (a) a physical property would have to be chosen which met the practical measurement criteria, and (b) in all likelihood the quantitative relationship between this physical property and water content would have to be established by us, taking into account the variations in the relationship which might be brought about by variations in the matrix material and water binding, and (c) whatever method was finally developed would have to be calibrated *in vitro* and water content from *in vivo* measurements would have to be inferred by reference to these *in vitro* calibrations. Our primary standard for changes in water content in the calibration specimens would be changes in weight, a direct measure of total tissue water, as this parameter was of primary concern to LAIR. We were also aware that much of the work would have to be done on areas where the stratum corneum is thick, the palms and the soles, so that samples of pure stratum corneum could be directly removed from the *in vivo* test site for subsequent *in vitro* calibration.

#### B. Thermal Constants

Our first approach was to examine the feasibility of using measurements of heat conduction constants of skin as a measure of its water content. Work by one of us had indicated that the thermal conductivity of the surface skin layers could be measured with a nonpenetrating method in which the skin surface temperature rise was recorded in response to a surface heat flux transient. Hydrated stratum corneum had a higher thermal conductivity than did dry stratum corneum (8) (Figure 1a). A feasibility study showed that the potential for this method was low (9), for the following reasons. First of all, the entire range of thermal conductivity from the dry to the moist state varied only threefold, and the variability was great (Figure 1b). Secondly, calibration measurements were slow, making it difficult to determine the *in vitro* relationship between water content and thermal conductivity. Thirdly, the simplified measurement scheme that we were using showed that there was as much dependence upon the dry mass or volume of the sample as there was upon water content. This problem, although correctable, would have resulted in a system of great expense and poorly adapted to *in vivo* measurement.

#### C. Electrical Characteristics

Measurement of electrical characteristics has proven to be

a more effective approach than the measurement of thermal constants. The measurement of electrical characteristics has been used industrially to determine moisture levels in powders, grain, and timber. Some basic work has been done on AC electrical characteristics of purified globular and fibrous protein powders in various states of hydration (10). Several investigators have approached the problem of inferring stratum corneum moisture levels by measuring changes in its electrical properties, but none have proposed a truly quantitative assay method (11,12).

### 1. Instrument Development

To simplify data analysis, we chose to measure surface electrical resistivity at a single very low sinusoidal frequency (1 Hz). Using a sinusoidal waveform partly overcomes electrode polarization effects, which are troublesome to DC measurements. To eliminate problems of contacting resistance, a four electrode method was chosen instead of the conventional two electrode design. In the four electrode method (long used in semiconductor research) the current source is connected to the outer fingers and the voltage-drop is independently measured across the inner two fingers with an extremely high input impedance buffer amplifier ( $10^{12} \Omega$ ). In this way the voltage drop accumulating across the current-carrying fingers because of AC polarization and contact problems does not result in an error in estimating the sample potential drop.

To insure that the resistivity ( $\rho$ ) measurement would uniquely reflect only the resistance of the stratum corneum and not the underlying tissue, it was necessary to design the electrode arrangement so that the distance between the current-carrying electrodes, (the outer two fingers), was less than the thickness of stratum corneum layer on which the probe would be used.

To meet all of these requirements, a state-of-the-art microelectrode was developed in conjunction the Microtechnology Laboratory at the University of Washington using microphotographic and etching techniques similar to those used in manufacturing integrated circuits. The probe consists of gold fingers evaporated onto a glass substrate (Figure 2). At the time of development, the best probe line resolution that could be obtained was 25 micrometer wide fingers spaced 35 micrometers apart, so that the applicability (in theory) was limited to specimens at least a quarter of a millimeter thick. This has suited our purpose to date, as we have needed to make measurements on sites of thickened stratum corneum which could then be removed for intensive *in vitro* study. Extension of these techniques to thinner stratum corneum may require the development of probes with smaller fingers and closer spacing.

Figures 3 and 4a show the electronic circuitry developed for use with the probe. The current source is of the constant current type with a selectable output in the nano-amp - micro-amp range. Extremely low current levels are used because of the close probe spacing. In addition, the voltage across the current carrying fingers is kept as low as possible as there has been some suggestion that the stratum corneum may exhibit non-linear resistance characteristics at different applied voltages (13).

Since the applied current ( $I$ ) is constant, the measured resistance ( $R$ ) can be determined from a strip chart recording of the potential drop ( $V$ ) across the inner two fingers. The material property of interest is *resistivity* ( $\rho$ ) which is equal to the measured  $R$  divided by a probe constant  $k$ . The value of  $k$  depends upon the width and spacing of the fingers. For our current probe design, the value of  $k$  is 4.53 mm. Our analysis has shown that  $k$  is also important in determining the theoretical minimum thickness of the sample upon which a valid measurement can be obtained. For the probes currently in use this thickness is 0.35 mm.

## 2. Accuracy of the Probe and Electronics

Special high accuracy resistors are appropriately interconnected and attached to the current source and voltage amplifier to simulate a four electrode measurement on materials varying in resistivity from 1 M $\Omega$ -mm to 1,000 M $\Omega$ -mm. Periodic calibration in this manner has shown the electronics to give accurate and reproducible results. In use, care must be taken to see that the constant current source is set to correct level. If the resistivity of the stratum corneum is extremely high because of low moisture levels, and the current source is set at too great of a level, then the current source pushes the limits of the power supply and clipping of the sine wave results. Under these conditions, of course, it is not possible to obtain a valid measurement unless the current level is decreased. Under these conditions decreasing the current level causes an additional problem, in that the potential drop  $V$  is decreased and the signal to noise ratio deteriorates, making it difficult to obtain an accurate measurement of  $V$ . With the addition of appropriate filtering and care in interpretation, we have found that the instrument can give valid information from about  $10^5$  to  $4-5 \times 10^8 \Omega$ . Resistances greater than  $10^8 \Omega$  are measured with less certainty.

Direct verification of a microelectrode probe have been and continues to be a problem. We have not been able to identify substitute materials for stratum corneum whose resistivity is in the 100 - 1000 M $\Omega$  range and accurately known, and which would present the same surface characteristics as skin to the electrode fingers. Therefore, direct calibration of the entire system in terms of accuracy of resistance measurement is not now possible.

We do not view this as a serious problem. The micro-probe is simply a mechanical extension of the electronics, wherein the real accuracy lies. The greatest potential problem with the four fingered probe would lie in mechanical inapplicability of the four-electrode method to this measurement set-up, i.e. four fine thin film lines applied to a fairly rough test site. If such mechanical problems existed they should show up as inability to obtain the same resistance values while repeatedly moving the probe on and off the same skin site. In addition, there should be a change in measured resistance with changes in application pressure of the probe. In our experience, however, these problems have not appeared. Identical values for resistance are obtained by successively moving the probe on and off the same site, and we have found that pressure loading past the point of making skin contact does not alter the measured resistance.

### 3. Quantitative Relationships Between Resistivity, Total Water Content, and Other Factors

At the outset of these studies there existed no quantitative data on the relationship between water content and resistivity for stratum corneum. The first step in developing a useful method was to determine how total water and other factors could affect the resistivity of stratum corneum material.

#### a. Test Methods

Samples of stratum corneum for *in vitro* study are obtained from the planter surface of the feet of normal volunteer subjects using a motor driven epidermal keratome fitted with a 0.4 mm thickness gauge. This procedure yields slices of pure stratum corneum approximately 1 cm x 1 cm x 0.3-0.4 mm thick. Sample dry weight is obtained after 48 hours of vacuum desiccation. At this point, the resistivity is extremely high, far higher than our instrumentation can measure. The samples are then reconstituted to different water contents and measurements are made at a test station consisting of a four-point probe mounted on a micromanipulator situated over a platform on the pan of an analytical balance (Figure 4b). Measurement of sample weight and resistivity can be made in rapid succession, and the probe can be applied with a uniform force measured with the pan balance. Total water content is calculated from the difference in weight at the time of the measurement and the weight after vacuum desiccation.

Two methods have been used for producing variable water contents in the specimens. The first is a modification of the desorption method described by Scheuplein and Morgan (14). In this method the dried samples are rehydrated by exposure for a fixed time period (24 hours) in a 100% RH chamber at room temperature. After this rehydration the sample is put on the balance pan and alternate weight and resistance measurements are

made as the sample dries out on the pan. Approximately four hours are required for the water content to decrease from a weight fraction of 0.7 (233% dry weight) to a weight fraction of 0.2 (25% dry weight), after which time the resistance has increased out of the measurement range.

The second is the equilibrium method, as described by Middleton, Spencer and others (15,16,17). Samples are equilibrated for 48 hours in sealed containers containing saturated solutions of various inorganic salts. These salts generate the following relative humidities: 60%, 75%, 83%, 90%, 93%, and "pure" water, about 98%. After equilibration, samples are put on the balance pan and a single measurement of weight and R is made; the sample is then put into another relative humidity chamber for re-equilibration to a different water content.

Both methods have advantages and disadvantages. The desorption method has the advantage of being more controlled, i.e. all resistance measurements are made on precisely the same small spot of the sample surface, and all are made within the same four hour time period. The desorption method has the disadvantage of generating changing gradients of water concentration through the sample as water is lost from the surface by evaporation, so that the water content by weight represents, at best, an average figure for the entire sample thickness and not necessarily the water content in the small volume of tissue under the electrode fingers.

The advantage of the equilibrium method is that theoretically it generates a uniform concentration of water throughout the sample, although this has never been validated. The disadvantages of the equilibrium method are that measurements are interspersed over many equilibration days, and there is much sample handling. It is not possible to precisely align the samples so that exactly the same microarea is seen by the probe fingers during each test.

#### b. Results With Normal Corneum

Both methods show a large reduction in R with increasing water content from a maximum  $R \sim 1,000 \text{ M}\Omega$  at a weight fraction of 0.25 (33% dry weight) to a minimum  $R \sim 5 \text{ M}\Omega$  at a weight fraction of 0.7 (233% dry weight).

Both methods show inter and intrasubject variability (Figures 5a, b). It is instructive to examine this variability. The well delineated curve patterns defined by changes in resistivity during desorption (Figure 5a) is partly due to the fact that there are many more data points during desorption than during equilibrium calibration. But also these well defined curve patterns indicate the high degree of precision with which the measurements

can be made (see esp. EM-21 and J-2), and show that for a single spot under the electrode fingers, there is a regular well-defined relationship between the total sample water and the resistance of that spot. Large discontinuities during a desorption experiment (i.e. A-2) can usually be traced to mechanical factors such as the sample sticking to the probe, movement on the pan, or changes in shape during the drying process.

On the other hand, variability between samples from the same or from different subjects appears much greater with the desorption method than with the equilibrium method. This may be due to real sample differences in the resistivity vs. water content relationship, but it is just as likely that the regional water content vs. total water content relationship was different during desorption in the several samples.

Examples of calibration data using the equilibrium method (Figure 5b) but on different samples, and different subjects than the desorption method show much less variation. Again, this could be due to a more homogeneous group of samples in terms of resistivity vs. water content relationship, but on the other hand such consistency would be expected if there was a more uniform state of hydration throughout the sample (which the equilibrium method is supposed to give). We are presently undertaking experiments in which both methods are used on the same samples. The data will give us some idea about the variability from sample to sample to be expected because of differences in regional vs. total water content during desorption. These experiments will not assure us however, of uniformity in hydration during equilibrium experiments.

#### c. Results With Pretreated Corneum

Other factors which could alter the relationship between resistivity and total water content might relate to the water binding characteristics of the stratum corneum matrix (22). Water which is tightly bound cannot fully participate in the conduction mechanisms as free water can. To test the influence of water binding and electrolyte concentration on the empirical relationship between resistivity and water content, the number of samples were twice tested, and before the second test were either (1) exposed formaldehyde vapor, which increases cross linking between stratum corneum proteins and therefore would increase from normal the ratio of the free water fraction to the bound water fraction at any given total water content (18), or presoaked in urea solution and then rehydrated. Urea supposedly increases water binding by its osmotic and keratolytical effects (19), and thus would decrease the ratio of free water to bound water at any given total water content.

The results are shown in Figures 6a and 6b. Formaldehyde exposure markedly altered the relationship between resistivity and water content by reducing the resistivity for any given water content. It was also evident that the rate of change of resistivity with changes in water content is much greater after formaldehyde treatment, and the total water pickup in 24 hours is less. (The desorption test method was used in these experiments). With urea pretreatment the relationship is altered by increasing the measured value of resistivity at each water content.

Lipid extraction and chloroform methanol solution followed by soaking in distilled water supposedly disrupts the structure of the cellular membranes and leaches out intracellular solubles (9,20). Samples soaked in physiological saline subsequent to this harsh chemical treatment (Figure 6c), dried and put through the standard desorption test, showed a depressed resistivity vs. water content characteristic relative to pretreatment. Lipid extraction is apparently crucial to this effect; soaking in distilled water or physiological saline without prior lipid extraction produces little effect.

#### d. Summary

Total water content appears to be by far the largest contributor to variations in resistivity in stratum corneum. Other factors contribute as well, but to a lesser degree. Physical chemical factors causing differences in water binding capacity between samples from different subjects may contribute to variations in resistivity. Variations in electrolyte distribution can also be a contributing factor.

Factors not related to the conduction mechanism may also be important. Differences in regional water content under the probe fingers may not always correspond with changes in total sample water content, particularly in the unsteady state desorption experiments. On the other hand, while the equilibrium experiments probably give more uniform results because regional water content is probably more closely related to total sample water content, the desorption studies may more closely parallel the *in vivo* situation since a gradient of water concentration is generated throughout the sample.

#### 4. Measurement of Resistivity of Intact Stratum Corneum

The probe is small enough so that it is possible to fasten it almost anywhere on the body surface, although our measurements to date have limited to the sole of the foot. Although the voltage probe amplifier has a high common mode rejection ratio it also has an extremely high input impedance so that noise pickup and stray capacitance can be a problem. For this reason each of the four electrode wires is connected by a separate shielded cable to the appropriate points in the constant current source and voltage probe amplifier. The shields are not grounded but driven to the same potential as the inner conductor; this procedure minimizes stray input capacitance.

It is essential in the operation of probe that the subject remain electrically "floating" at the time of measurement, and not be in contact with earth, the DC power supply, or any large metal objects.

Large muscle movements can sometimes set up enough EMF to distort the waveform but this is usually of a very transient nature and in our application has not posed a serious problem.

Measurements can be made as soon as the probe is in contact with the skin; the only electrical settling time for the instrumentation is the one second time lapse for a complete one hertz waveform. The occlusive nature of the probe allows us to alter the water content of the stratum corneum during measurement of resistivity by simply leaving the probe on the skin surface. Because the stratum corneum is relatively impermeable to the transport of water vapor, initially the concentration gradient of water across the surface layer is extremely steep resulting in a very dry skin surface under normal conditions. When the probe is put on the skin surface the concentration of water in the surface layers immediately begins to change rapidly, but it is several seconds before concentration in these surface layers increases to a point where the resistivity change can be measured (weight fraction equal to 0.2, 25% dry weight). A mathematical model of this diffusion boundary value problem has been presented elsewhere (21,22). Eventually, of course, the water content under the probe will come into equilibrium with the underlying tissues. The time constant of this equilibration process is  $\tau$ , and the value for  $\tau$  can be computed from:

$$\tau = \frac{4l^2}{\pi^2 D} \quad (\text{Equation 1})$$

$l$  is the thickness of the stratum corneum layer,  $D$  is the diffusion coefficient for water in stratum corneum. Figure 6d is a plot from the solution of this boundary value problem using a value of  $\tau$  equal to 16 minutes.  $\tau$  was calculated on the basis of  $l = 0.15$  mm and  $D = 9.3 \times 10^{-8}$ -sec<sup>-1</sup> (23). With occlusion, changes in water concentration are much more rapid at the surface than any where else in the stratum corneum. They occur most rapidly during the initial stages of occlusion. Coupled with this is the fact that incremental changes in water content produce much larger changes in resistance at low water contents than at high water contents. Therefore, upon application of the four fingered electrode to the intact skin surface, one would expect a very rapid decrease in resistance during the first minutes of occlusion and that the change in resistance would gradually become much less at extended times as equilibrium is approached. (At this point, it is worth while to point out that Figure 6d with different values for  $l$  probably is a fair representation of changes in concentration of water seen during the desorption experiments except that during desorption, zero depth indicates

the center of the hydrated sample and water concentration gradients would extend from the center to both sides.)

Figure 7 shows the result from a typical evaluation experiment where a small skin area on the bottom of the foot was first conditioned by exposure to a dry nitrogen gas stream at 2 l/min. for 20 minutes. Then the probe was applied to this area and left on for an additional 40 minutes. As predicted, initial resistivities decreased quite rapidly from about 130 M $\Omega$  after 2 minute of probe occlusion to about 16 M $\Omega$  at 20 minutes. Thereafter the rate of decrease was considerably less falling to only about 12 M $\Omega$  by 40 minutes. It should be emphasized that the range of resistivity obtained over this 40 minute period reflects the degree of dryness at the start and the change with transepidermal water loss over the 40 minute period. If the skin were pre-hydrated, by occlusion or by soaking one would not expect to see this large change in resistance.

#### 5. Proposed Application Method for Inferring *in vivo* Water Content From *in vivo* Measurements of Resistivity

There are a number of factors which rationally prevent the application of a *general* calibration curve to a *specific* measurement of resistivity *in vivo* as shown in Figure 7. These factors are (1) a lack of complete understanding of the mechanisms of electrical conduction, and factors which affect these mechanisms, as they might vary from sample to sample and subject to subject, and (2) uncertainty about how the total water content of a sample during *in vitro* calibration reflects the water content of the areas seen by the four-fingered electrode.

In spite of this, we feel that the four fingered electrode method can now be used with reasonable accuracy in studies to measure the *in vivo* water content of stratum corneum if the following procedures are followed.

- (1) The measurements must be confined to the thickened stratum corneum of the palms or the soles.

- (2) The *in vivo* area on the palm or sole in which it is of experimental interest to measure water content or changes in water content must be carefully marked beforehand and the four fingered microelectrode applied within this marked area during the experimental procedures. This procedure will allow removal of the same test area for subsequent calibration. This procedure should assure that differences in the electrical conduction characteristics because of differences in water binding capacity or electrolyte concentrations will be

minimized. Each sample will serve as its own control.

(3) At the conclusion of *in vivo* experimentation the test site must be removed for individual calibration. To do this we use a Storz type epidermal keratome fitted with a 0.4 mm thickness gauge. We have found it necessary to attach a more powerful motor to the keratome to cut plantar corneum than was originally supplied with the instrument. At this point the sample can be dried and stored until such time as *in vitro* calibration is possible.

(4) At the time of *in vitro* calibration the sample should be cut so that the marked area of measurement is in the center and excess material is trimmed away. Exactly how large the remaining calibration sample should be is a technical question whose answer will depend on methods available to the calibration laboratory. Making the sample very small has the advantage of making regional water content, under the area seen by the probe, nearly the same as total water content in the sample. Making the sample too small will make water losses from the perimeter more significant, a problem which is not encountered *in vivo* and which can be ignored when the total sample area is much greater than the thickness. Practical limitations are imposed by the accuracy and sensitivity of weight measuring apparatus and the ability to process and handle extremely small fragments of tissue without damage.

(5) The advantages and disadvantages of the desorption method and equilibrium method for obtaining calibration curves of resistivity vs. water content has been discussed. While the desorption method has the advantages of a closer similarity to the *in vivo* water concentration gradient and giving many more data points than the equilibrium method it has the theoretical disadvantage of showing a poorer relationship between regional water content and the total water content of the sample measured experimentally. The water concentration gradient from the surface to the maximum depth of measurement of the electrode is nearly flat for a large number of water concentrations anyway (Figure 6d). It is probably wise at this time, if the sample is large, to choose the equilibrium method. Although the equilibrium method takes a number of days to secure

completion of the calibration, a large number of samples can be processed at the same time so that the total technician time is considerably less than for the desorption method. We are currently conducting direct comparison of the two calibration methods on the same samples. This information should help us arrive at a more definitive recommendation in the future.

Calibration data so obtained should provide the user with a method of reasonable accuracy for translating measurements of *in vivo* resistivity made during experimental procedures to estimates of total water content of that spot during the procedures. In this method resistivity is not used as a *direct* measure of water content *in vivo*, because of differences in conduction mechanisms, water binding, and electrolyte concentration which might exist between subjects. Rather, resistivity is used as a way to transfer information between the *in vivo* situation of unknown water content and the *in vitro* situation of controlled water content. In this respect we have shown that resistivity is an easily measured, accurate and reproducible *transfer measurement*. The assumptions required are (1) that in the process of transfer of the tissue from the *in vivo* to *in vitro* situation the relationship between resistivity and the physical chemical properties of the stratum corneum do not change, and (2) that the changes in total water content of the sample as measured by weight change reflect the changes in regional water content under the probe. The first assumption is one of long standing in investigative dermatology; nearly all of the published work relies on the assumption that removal of this outer dead keratin layer in no way affects its physical chemical properties (4,5,7,8,14,15,16,17,18). The second problem is one on which there is no direct evidence, as it is an empirical problem, related only to the interests of this study, and one which deserves more exploration.

#### 6. Results From Human Experiments Illustrating the Use of the Proposed Application Methods

We are conducting a number of experiments to illustrate the application of this method and to define problems encountered in its use. These are ongoing studies and not yet concluded; we present here the results to date.

In these experiments we were interested in seeing the range of change in resistivity and subsequent water content which could be obtained during a forty minute period of occlusion of transepidermal water loss from the skin surface. Five male and three female adult volunteers were used as subjects. The same area on the sole of the forefoot was used on all subjects. The four fingered microelectrode was mounted on a plunger inside

of a cup through which dry nitrogen flowed at 2 l/minute. Pushing the plunger brought the microelectrode in contact with the test site. Each test occlusion and resistivity measurement period lasted 40 minutes; these 40 minute periods were interspaced with 20 minutes of nitrogen dry-down with the plunger pulled back from the skin surface. Three dry down periods and three measurement periods constituted an experiment for an individual subject lasting 180 minutes. At the conclusion of the *in vivo* experimentation, the test site was removed with the epidermal keratome using a 0.4 mm thickness guage. Samples were trimmed, desiccated and the dry weight obtained. Calibration of the test site *in vitro* was obtained with the equilibrium method at 60%, 75%, 83%, 90%, 93%, 97%, and 100% relative humidity. The results are shown in Figures 8 - 15. All of the subjects showed essentially the same kind of response by the second or third test period, i.e. a very rapid initial decrease in resistivity followed by a slower change in resistivity toward the end of the 40 minute occlusion period. More variation was seen in the response during the first occlusion period, and this may be related to initial prehydration effects from foot wear which were not fully compensated for by the initial 20 minute dry down period. Six of the eight subjects showed remarkably linear individual calibration curves of log resistivity vs. total water content. Data from two of the equilibration relative humidities, 60% and 75%, never appeared in the final calibration curves as the water pickup at these relative humidities (10 and 12% dry weight respectively) was too low to produce any measureable change in resistivity. Of the remaining relative humidities, 83% produced an average water content of 29% dry weight and a resistance never lower than 600 M $\Omega$ , while 100% relative humidity yielded an average water content of 288% dry weight and the resistivity which was never greater than 10 M $\Omega$ . The first *in vivo* observations were made one minute after placement of the probe, so that the initial very rapid phase of falling resistance is not shown. In practice we attempt to connect the calibration points with a best fit line over the range of resistances observed in the *in vivo* experiments. This was no particular problem in 6 of the 8 subjects where a straight line could easily be drawn. For the other two subjects curve fitting was more difficult, and we chose to connect the equilibration points rather than draw a straight line fit through them. No technical reason could be found for discounting the data of these two equilibration experiments, and at this time there is no theoretical justification for assuming that all subjects from all samples should show a straight line relationship. For this reason, we recommend that the individual calibration procedure be followed.

## 7. Recommendations for Future Research

We feel that the four line microelectrode method for measuring skin resistivity has proven to be a reasonable transfer method. It meets all the initial criteria for selection of a method, with which data on the time course of change of measured physical property can be made during experimental manip-

ulations and later converted into estimates of water content by direct calibration of the sample under known conditions. In its present application, the procedure for obtaining the sample from the foot of the volunteer subject is simple, fast and painless, and in this regard is not unlike many other clinical tests which require samples of blood or tissue for their completion.

We feel that the major area for improvement of the transfer method lies in improvements in the calibration procedure. Specifically, we recommend studies to determine how regional water content in a small area under the probe varies in relation to the total sample water content, and miniaturization of the calibration technique to minimize errors of this kind.

At this time we lack a firmly proven model to explain the physics of electrical conduction processes within the stratum corneum, although we feel that major contributory factors have been identified. Whereas in the *transfer method* such factors can be overlooked, as each sample serves as its own control, in an *absolute method* relating total water content to some external resistivity standard, variations in conduction mechanisms must be taken into account.

It is in the interest of our contract research but beyond its scope to investigate further the basis of conduction mechanisms and frequency dependent effects in stratum corneum. For this purpose, Dr. Eugen Schibli of our laboratory and a coinvestigator on this contract has submitted a grant application which is pending with National Institutes of Health. Dr. Schibli's application deals specifically with the question of conduction mechanisms and is not directly concerned, as is this contract, with the development of empirical methods. The results of Dr. Schibli's basic studies should be of great value in interpreting our present data and in designing future improvements.

We also recommend using the present probe and new closer spaced probes in further studies on thin stratum corneum making direct comparisons with thickened stratum corneum with the view that eventually these empirical techniques might be extended for use on the whole body surface.

Some detailed mapping of variation in resistance patterns in small skin areas on different parts of the palms and soles should be undertaken to determine the kind of variation normally seen *in vivo*. Such studies would give an indication of expected variation from different sample sites on the same subject.

Finally, we recommend that the method, as it is now, be incorporated into planned or ongoing studies in which changes in plantar or palmar water content or hydration characteristics is a major factor. Using the probe in such studies might not only benefit those studies but would give us additional

information necessary for refinement of calibration and application techniques.

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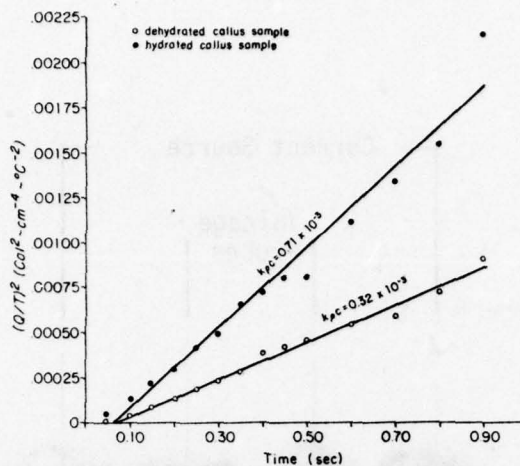


FIGURE 1a: Initial observations on kpc in dry and hydrated callus.

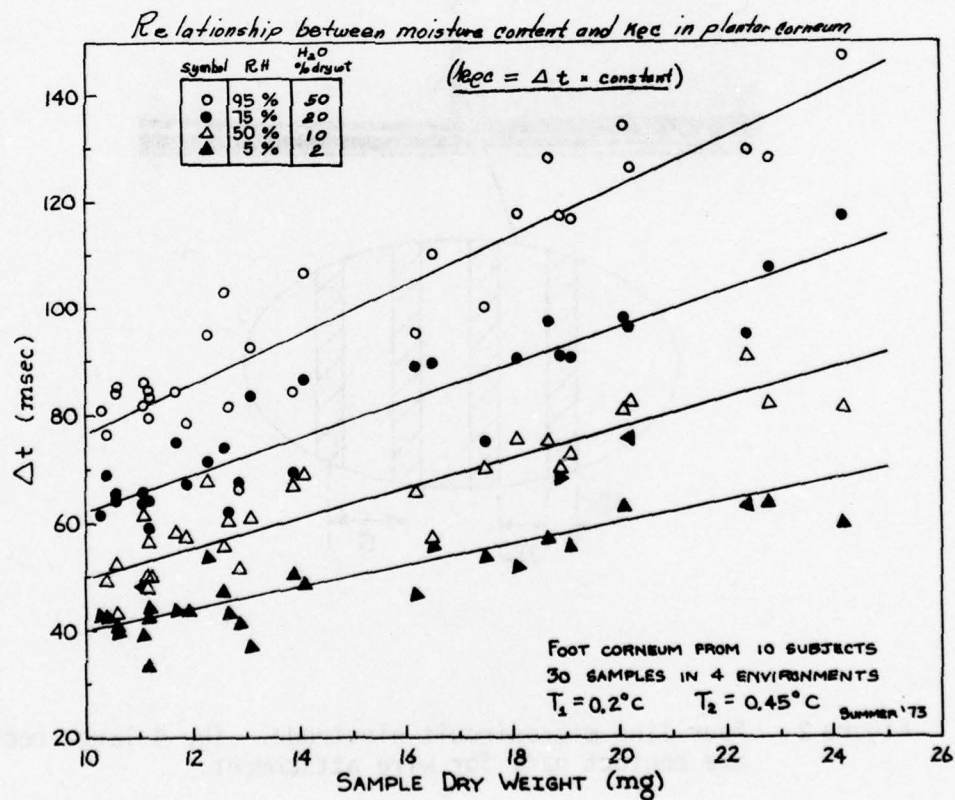


FIGURE 1b: Results of pilot studies to quantitate relationship between water content and kpc

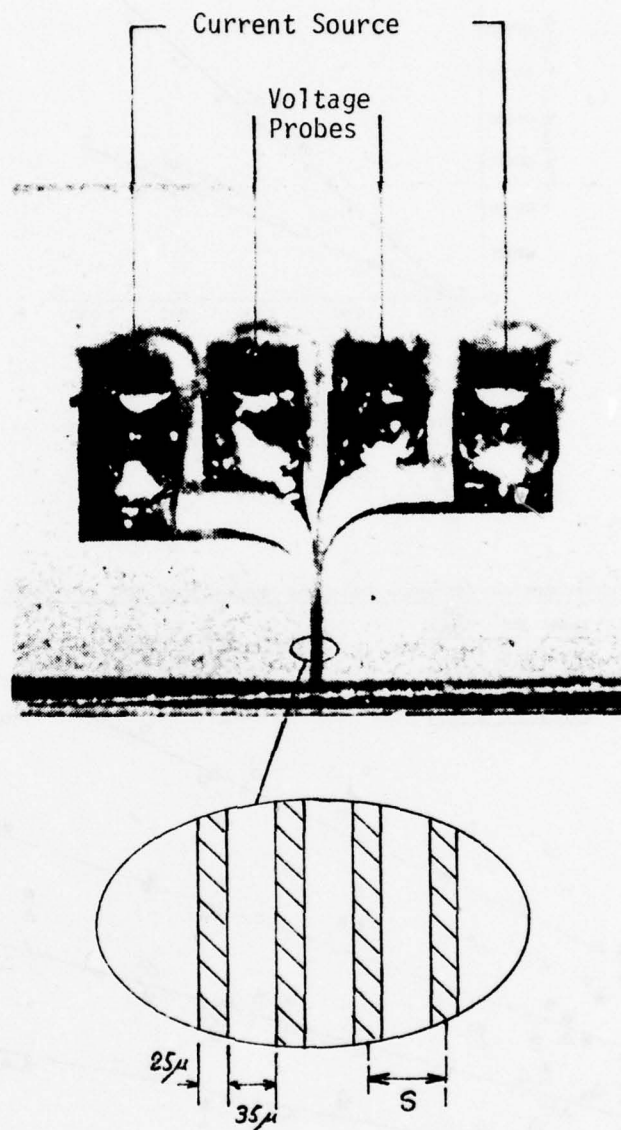
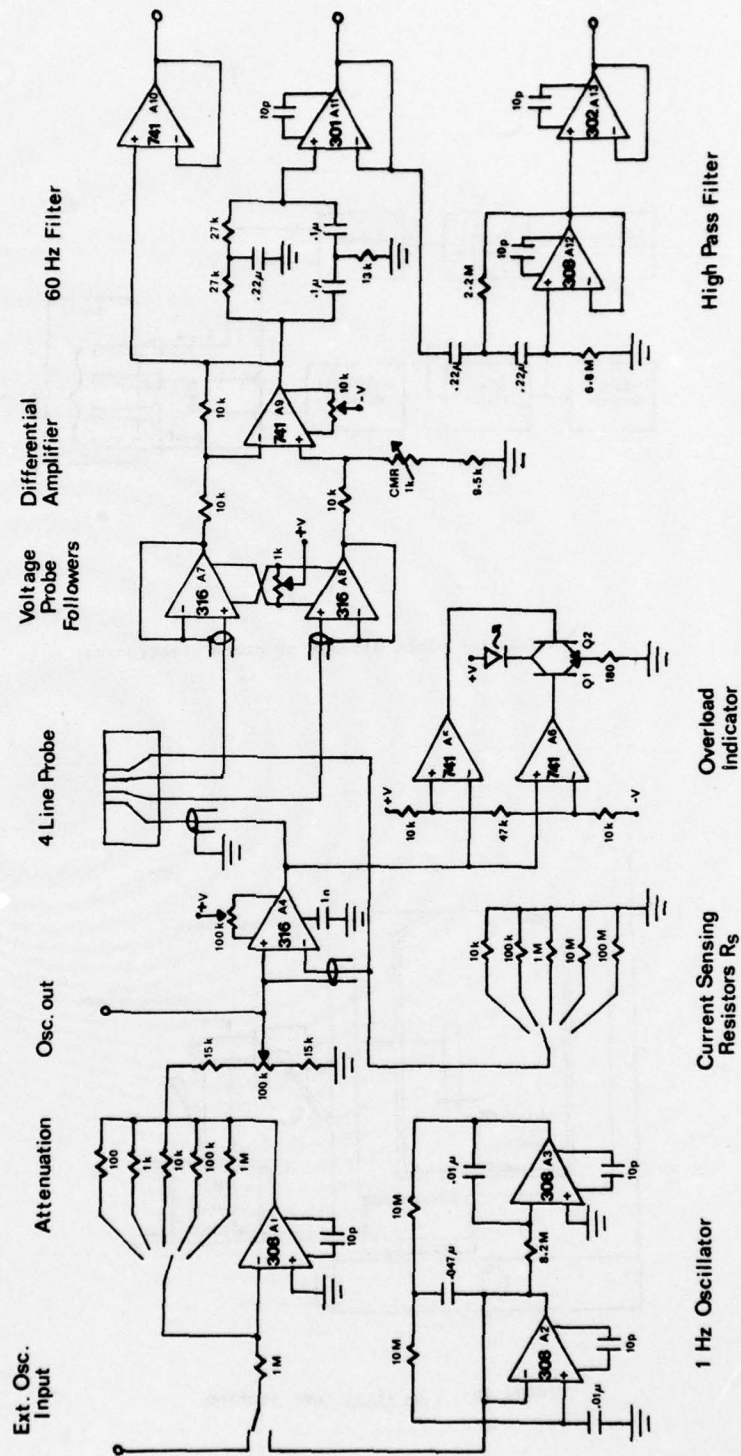


Figure 2. Four-line microcircuit electrode. The 4 large rectangles are contact pads for wire attachment.



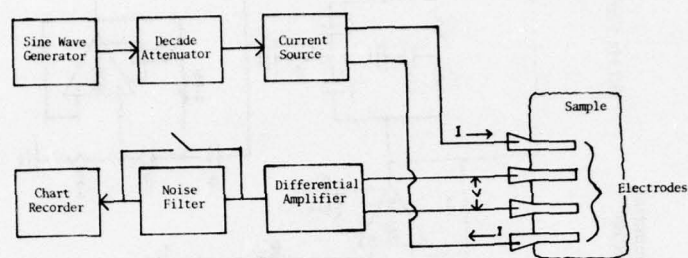


FIGURE 4a: Block diagram of probe electronics

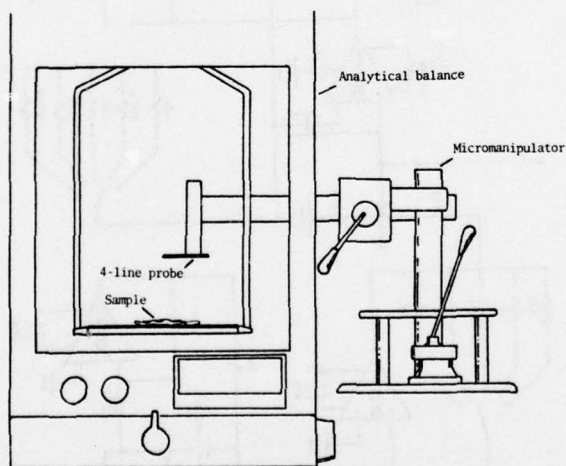


FIGURE 4b: In vitro test station

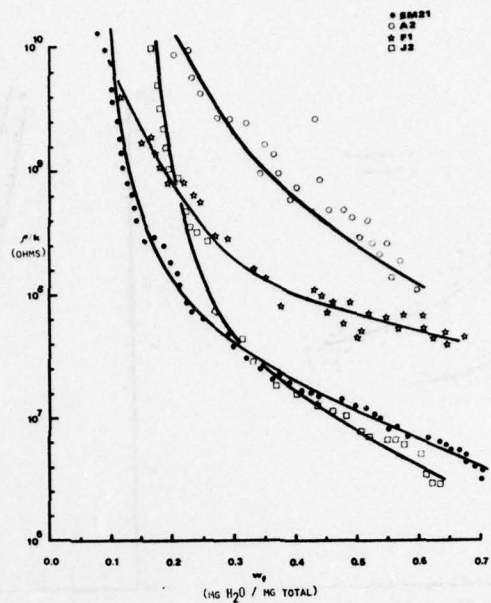


FIGURE 5a: In vitro test results from five subjects using desorption method

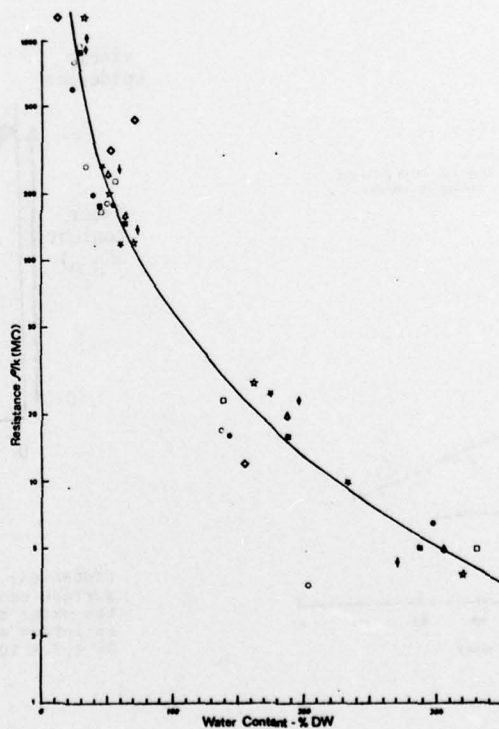


FIGURE 5b: In vitro test results from nine subjects using equilibrium method

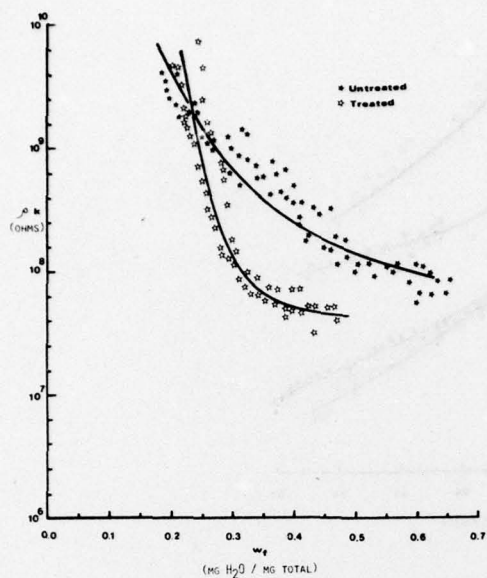


FIGURE 6a: Resistivity vs water content before and after formaldehyde treatment

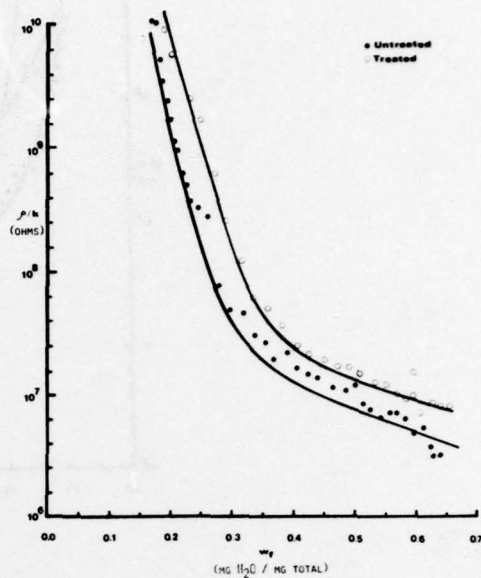


FIGURE 6b: Resistivity vs water content before and after urea treatment

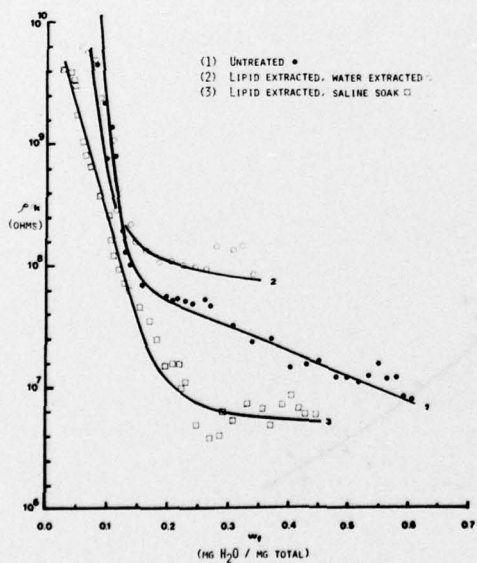


FIGURE 6c: Resistivity vs water content before and after lipid extraction followed by distilled water and saline soak

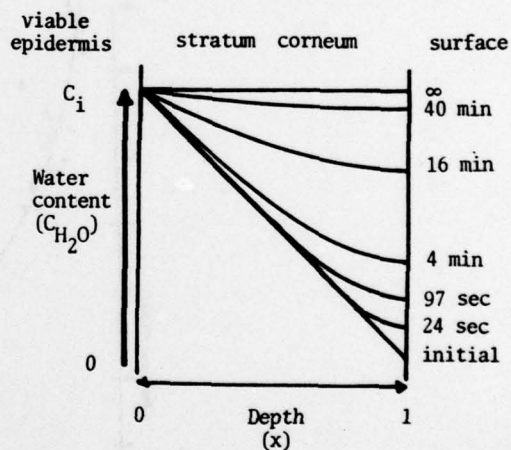


FIGURE 6d: Calculated effect of continued surface occlusion on relative changes in the water concentration gradient with time in intact stratum corneum. Assumed  $l=0.15$  mm,  $D=9.3 \times 10^{-10}$  cm<sup>2</sup>-sec<sup>-1</sup>,  $t=16$  minutes.

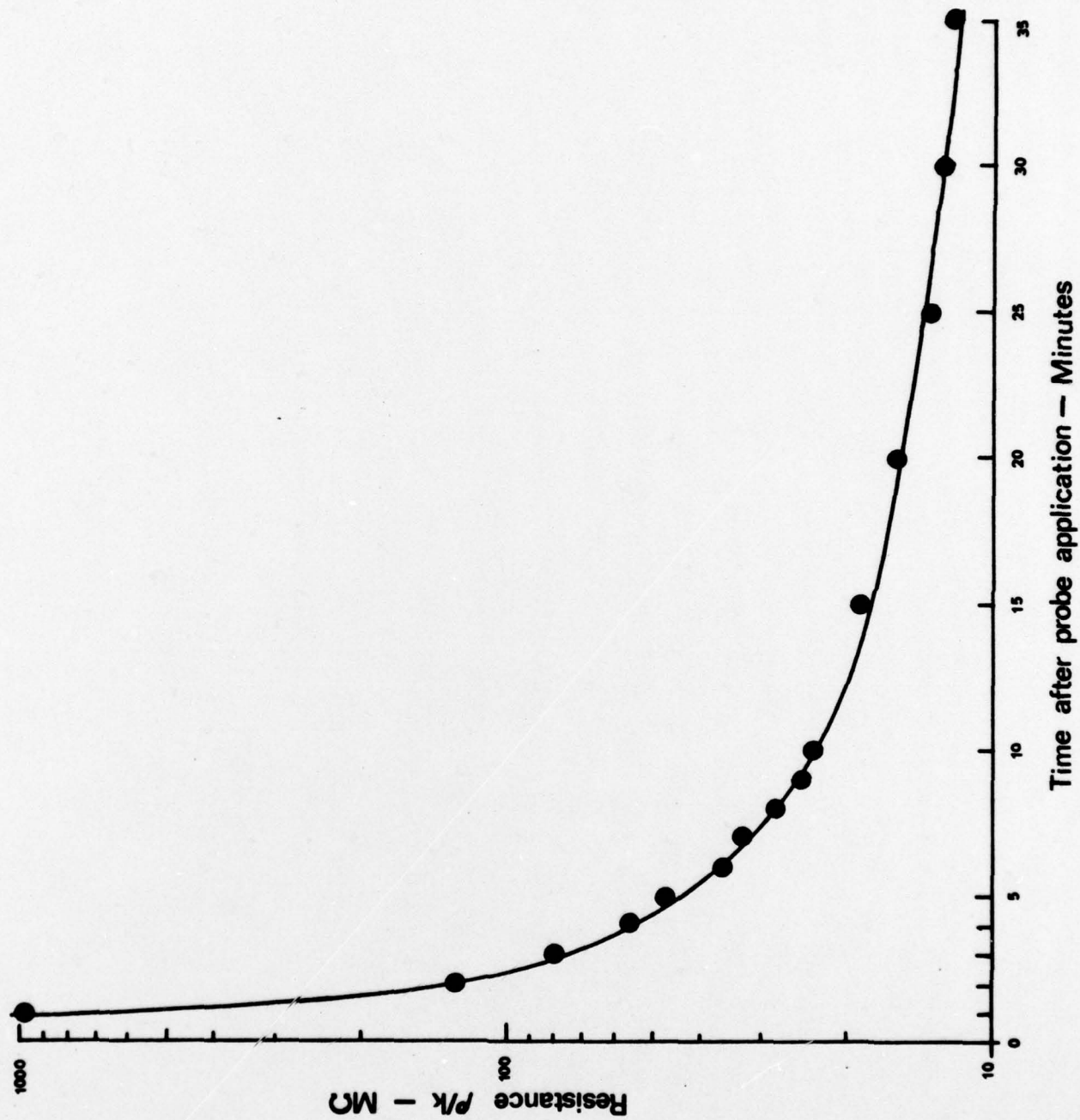


FIGURE 7: Selected in vivo application curve to show occlusive effects of probe on electrical resistivity

LEGEND FOR FIGURES 8-15

Figures 8-15 are the results of studies on eight different subjects to illustrate the use of the resistivity transfer method for estimating water content in vivo. There were three periods of probe application each lasting forty minutes. Prior to each measurement period the test site was dried for a twenty minute period with a flow of dry nitrogen gas at 2 L/min. Following the study, a 0.4 mm thick section of the test site was removed for calibration with the equilibrium method.

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UPPER LEFT: Time course of resistivity changes at test site during in vivo study

UPPER RIGHT: Relationship between resistivity and water content at test site during in vitro calibration experiments

LOWER: Derived time course of water content during in vivo study

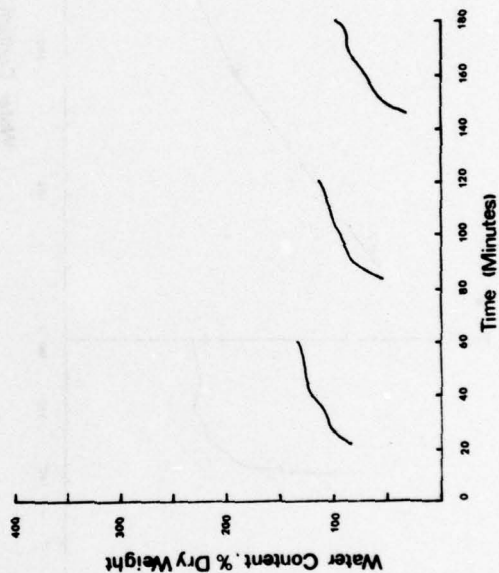
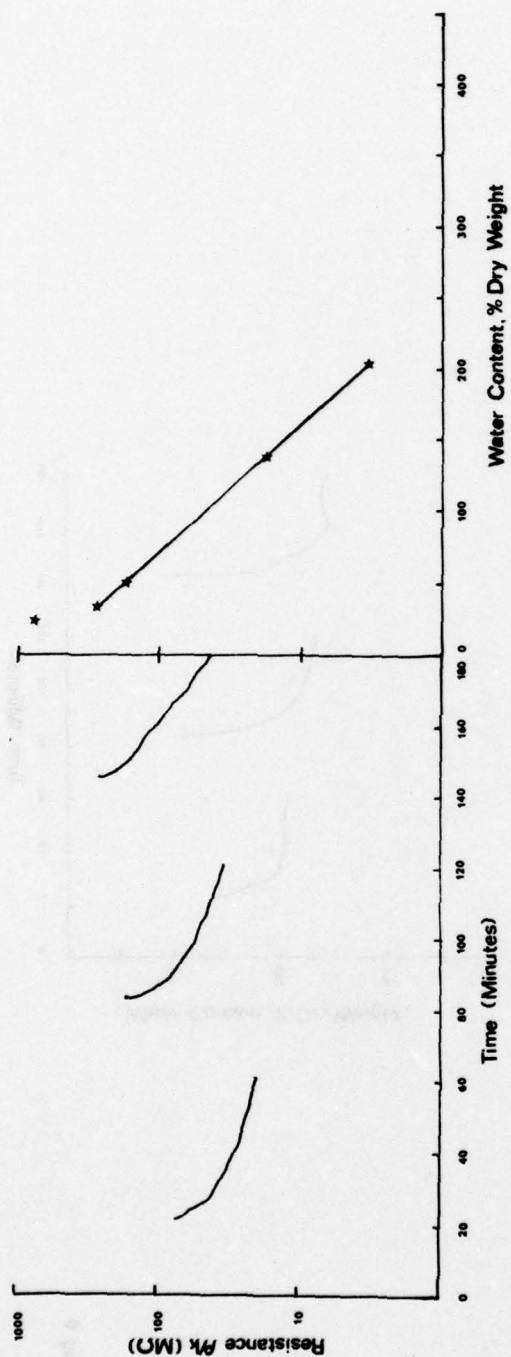


Fig 8

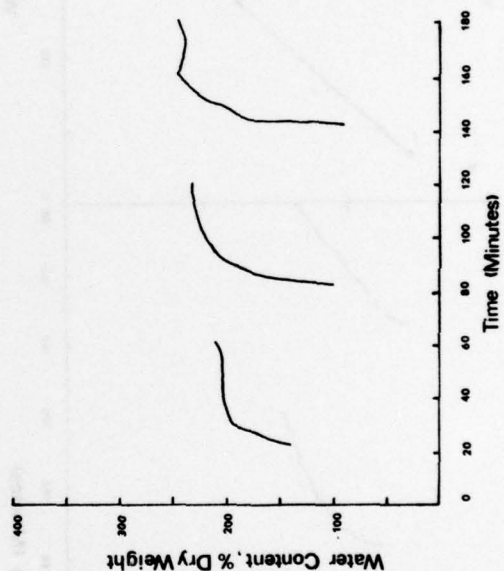
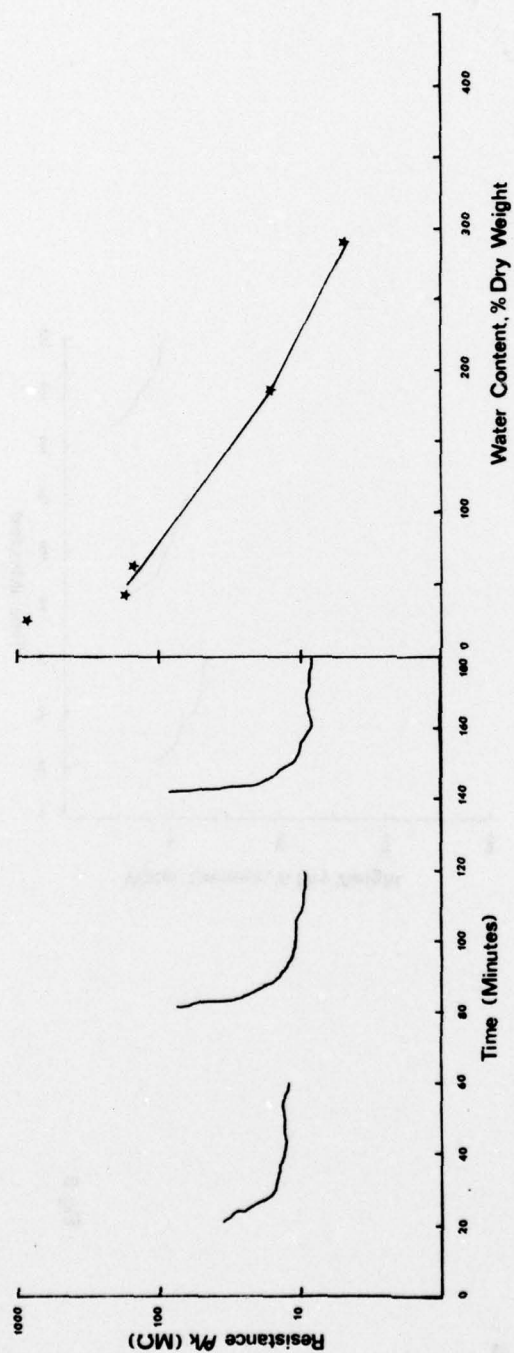


Fig. 9

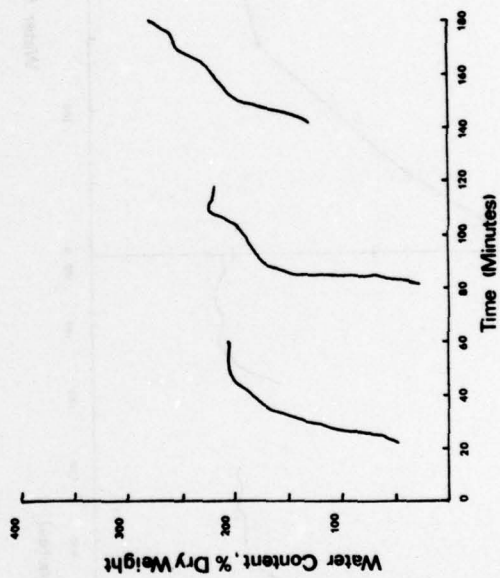
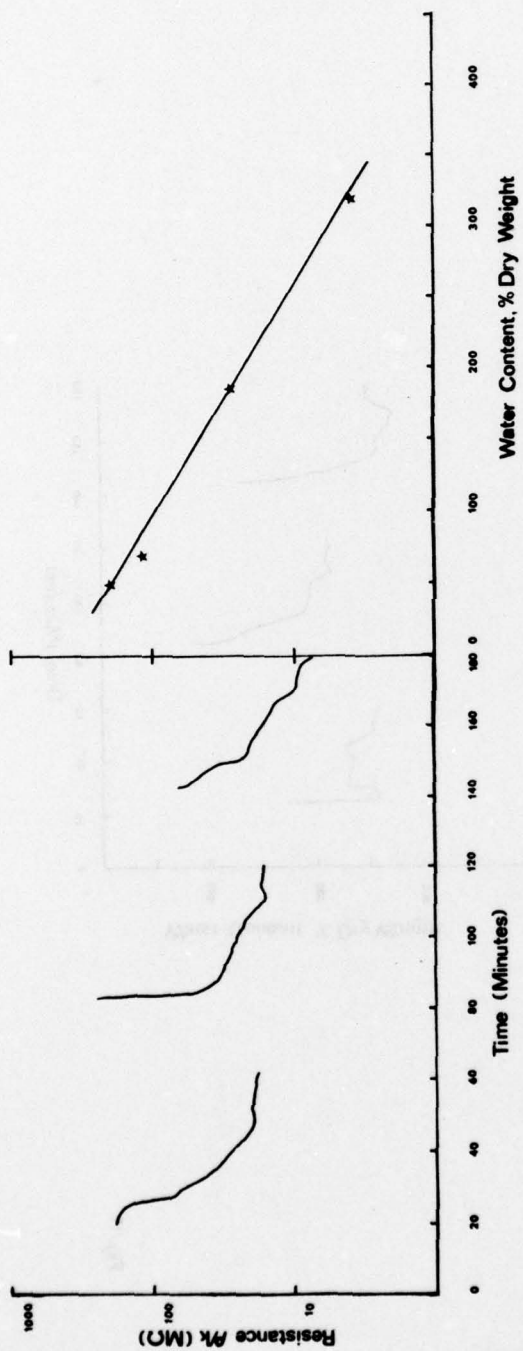


Fig. 10

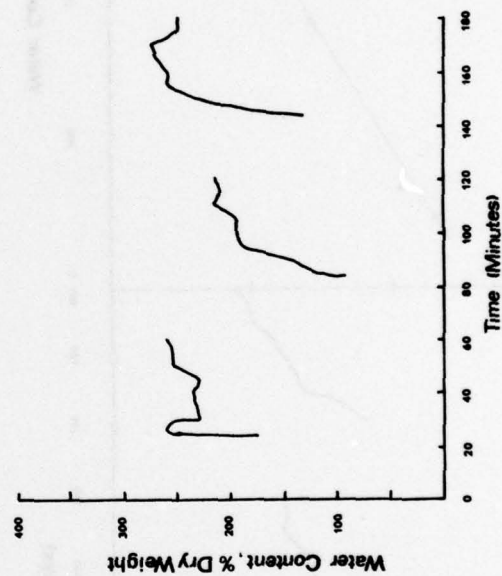
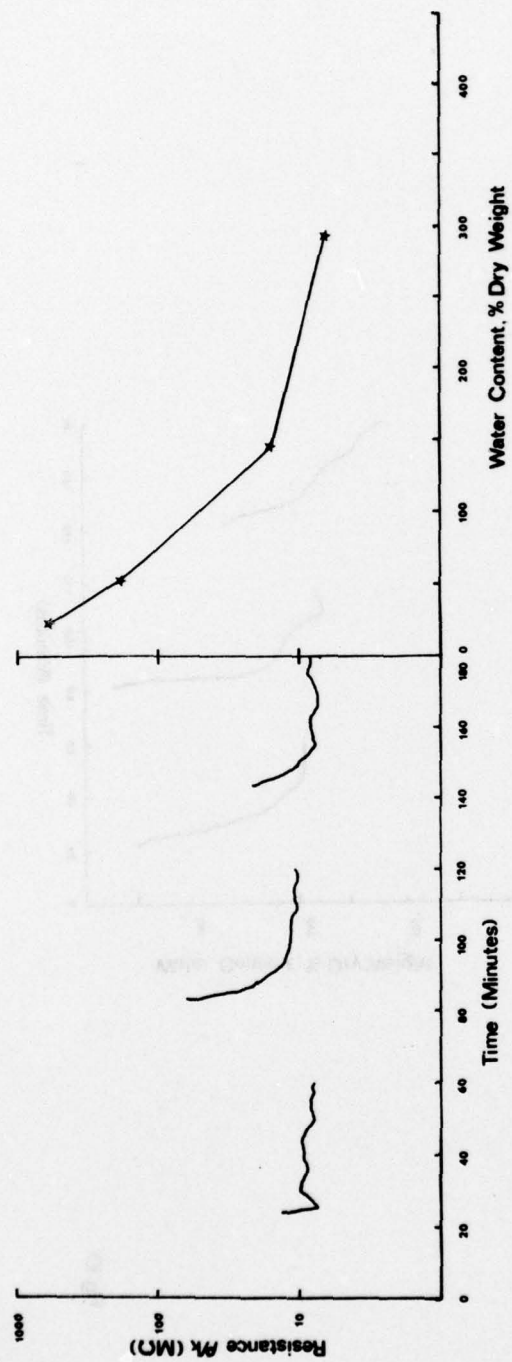


Fig. 11

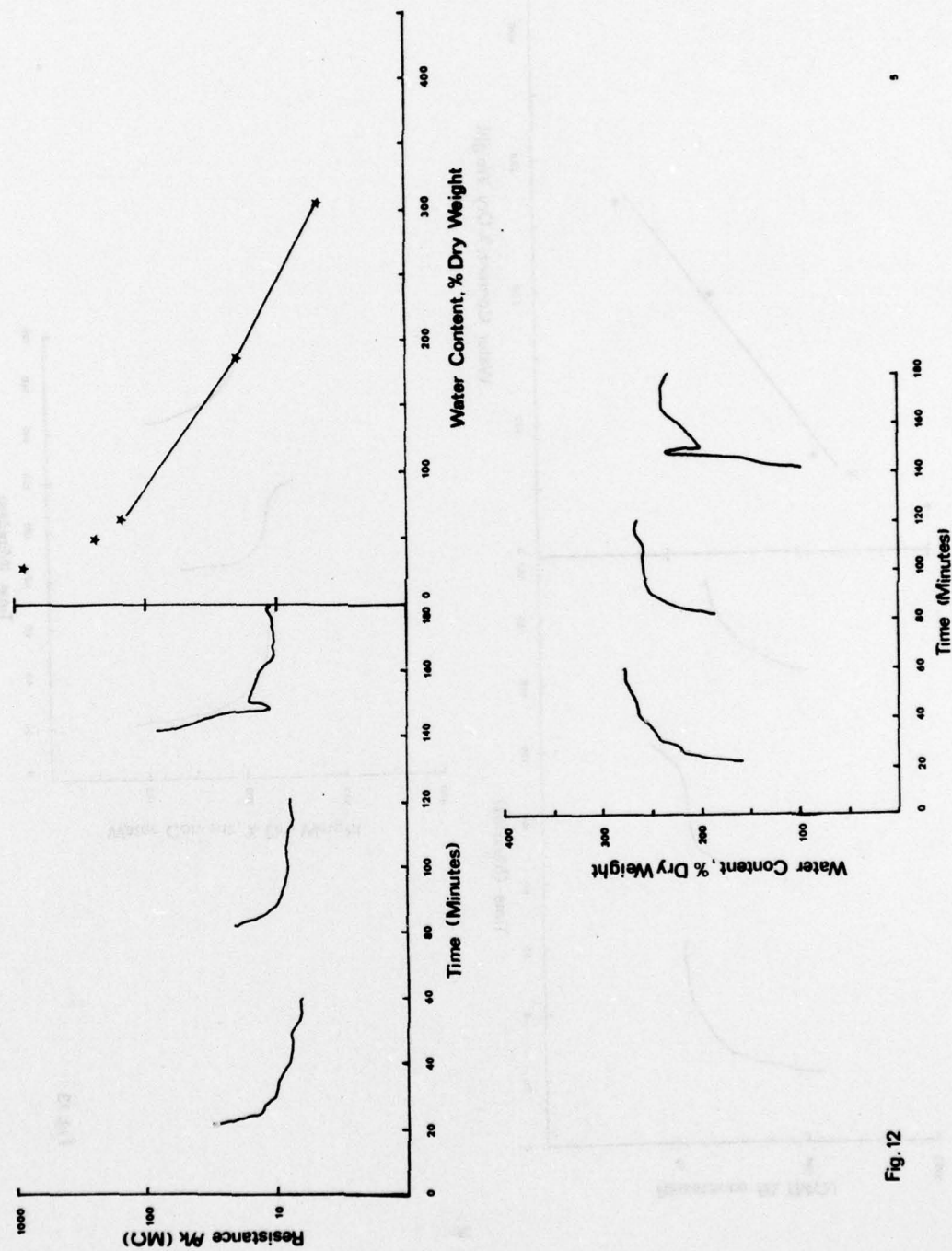


Fig. 12

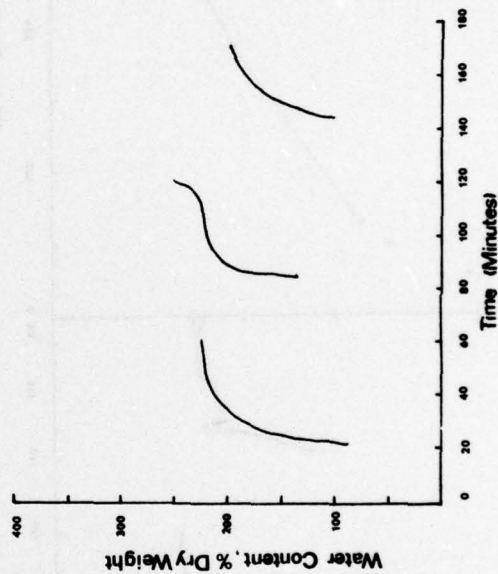
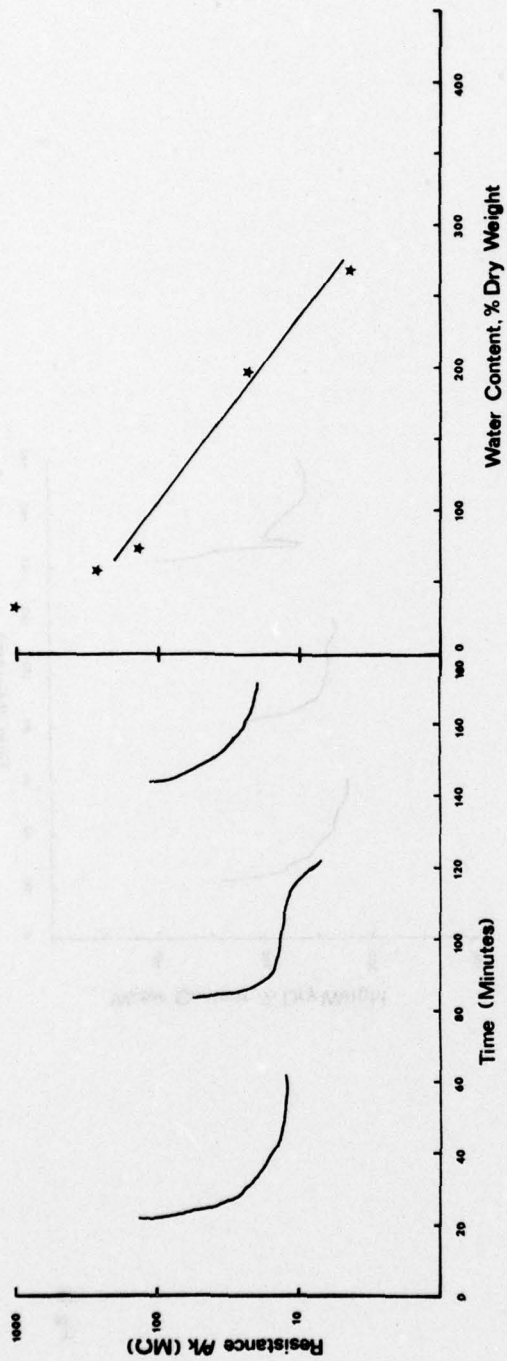


Fig. 13

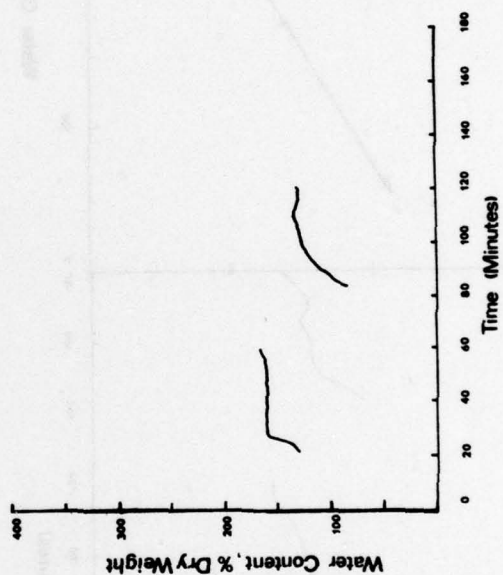
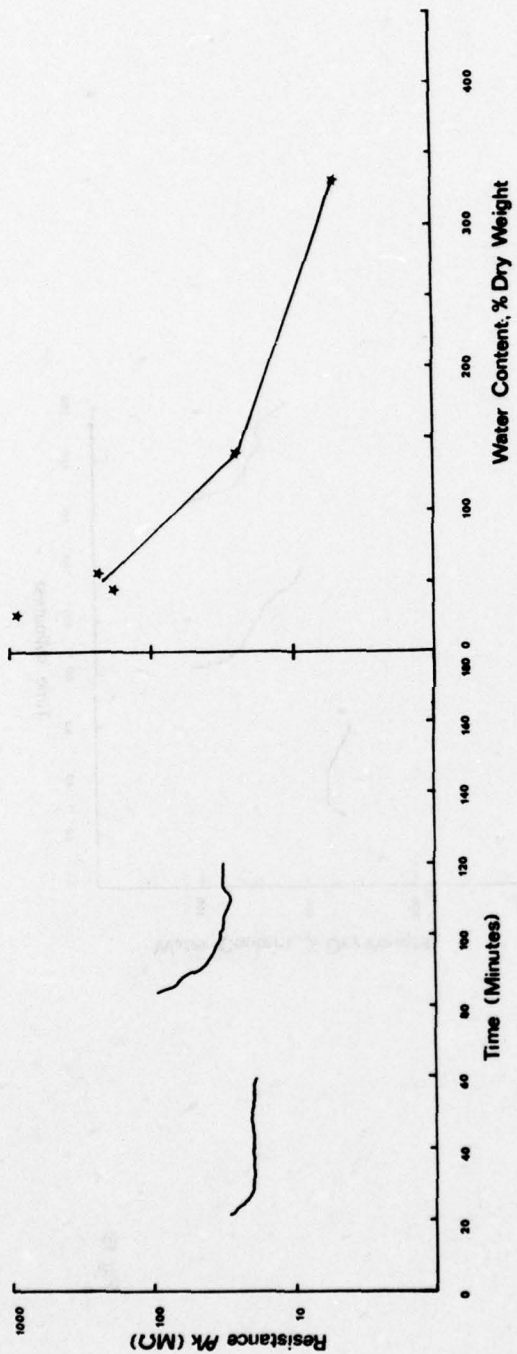


Fig. 14

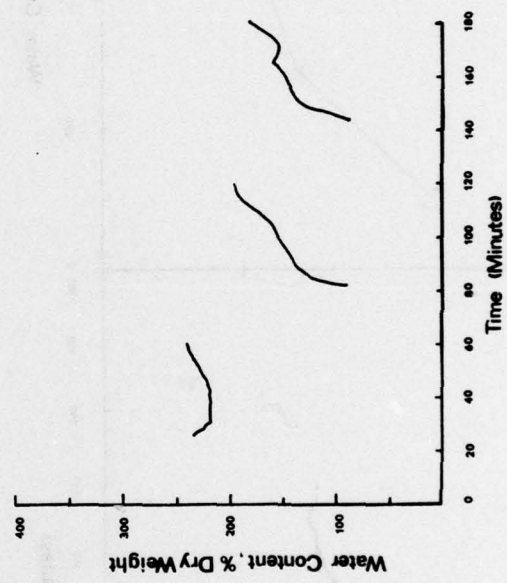
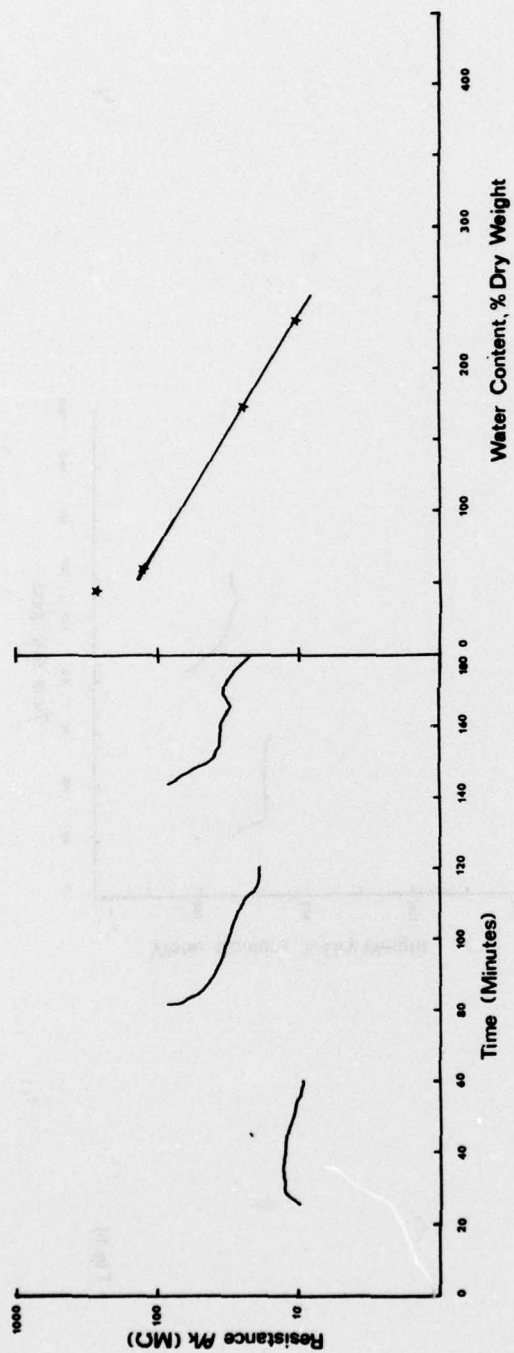


Fig. 15

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